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THE ORIGIN OF THE NERVOUS SYSTEM IN *PENNARIA TIARELLA*, AS REVEALED BY TREATMENT WITH COLCHICINE*

VICKI JOAN MARTIN[1] AND MARY BETH THOMAS[2]

Wake Forest University, Winston-Salem, NC 27109

Abstract

Treatment of 8-h embryos of the marine hydrozoan *Pennaria tiarella* with colchicine for 2 h eliminates interstitial cells, nematoblasts, and ganglionic cells. Comparisons of interstitial-cell-less and control planulae of *Pennaria* by TEM and light microscopy suggest a dual origin of the nervous system in *Pennaria*. Ganglionic cells are shown to be derived exclusively from endodermally-derived interstitial cells, whereas neurosensory cells are derived from cells of the ectodermal epithelium, probably from the epitheliomuscle cells. This investigation supports the idea that ganglionic cells and neurosensory cells can differentiate not only from different stem cells but also from different germ layers.

Introduction

Cnidarians are considered to be the first multicellular organisms in which a nervous system evolved (Davis, 1973). The origin of the various types of nerve cells in the cnidarians, namely, neurosensory, ganglionic, and neurosecretory, has been described in detail for *Hydra*. Generally, it is believed that all nerve cells arise from an embryonic reserve cell, the interstitial cell (McConnell, 1932; Burnett and Diehl, 1964; Lentz, 1965; Davis, 1969, 1972). The origin of nerve cells in cnidarians other than *Hydra* has not been reported.

A recent ultrastructural analysis of the nerve elements of the planula of the marine hydrozoan *Pennaria tiarella* (Martin and Thomas, 1980) identified two types of nerve cells. Type I nerve cells are at the base of the ectodermal epithelium just apical to the forming foot processes of the epitheliomuscle cells. Neurites projecting from these Type I nerve cells form a nerve plexus of transversely and longitudinally oriented processes. These Type I nerve cells resemble the ganglionic and sensory-motor-interneurons described for *Hydra* (Davis, 1971; Westfall, 1973). Type II nerve cells are restricted to the epidermis and are morphologically similar to the neurosensory cell described by Davis (1969) for *Hydra*.

The present study examined the origin of the nervous system in *Pennaria*. Colchicine was employed during embryonic development to produce planulae devoid of interstitial cells. These planulae were then examined for nerve cells by light and electron microscopy.

Materials and Methods

Mature colonies of *Pennaria tiarella* were collected in August 1979 from wharf pilings in Morehead City, North Carolina. Fronds from male and female colonies

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[1] Present Address: Department of Zoology, University of Alberta, Edmonton, Alberta, Canada T6G 2E9.

[2] Present Address: Department of Biology, University of North Carolina at Charlotte, Charlotte, N. C. 28223.

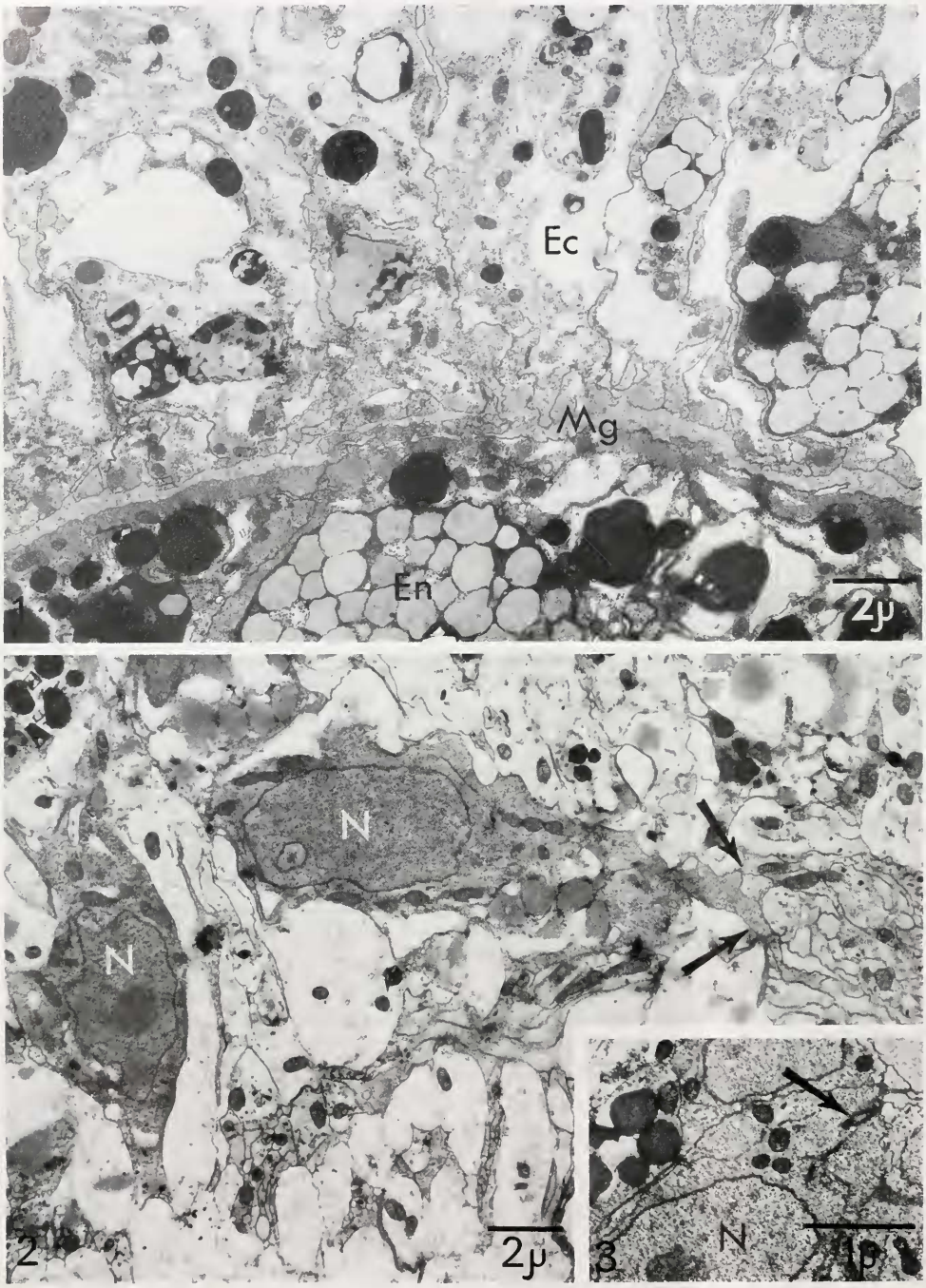

FIGURE 1. Transverse section of a colchicine-treated planula which has recovered for 72 h. Ganglionic cells are absent in these larvae. Ec = ectoderm; En = endoderm; Mg = mesoglea. Bar = 2 μm.

FIGURE 2. Transverse section of a region of a control planula comparable to the region of the colchicine-treated planula in Figure 1. Neurites (arrows) radiate out from the ganglionic cells, which occur between the developing foot processes of the epitheliomuscle cells and the nuclei of the ectodermal

were placed together at 5:00 p.m. in large finger bowls of filtered seawater. The bowls were placed in the dark at 6:00 p.m. At 9:00 p.m. early cleavage stages were removed to filtered seawater until 8 h after fertilization (3:00 a.m.).

The 8-h embryos were treated for 2 h in 0.4% colchicine (Sigma Corporation) dissolved in filtered seawater. The embryos then were washed repeatedly and placed in small finger bowls of filtered seawater. After a 12-, 24-, or 72-h recovery period in reagent-free normal seawater, experimental and control planulae were prepared for transmission electron microscopy (TEM) and light microscopy. Untreated and treated planulae were also examined at 48 h after fertilization.

Planulae to be examined by TEM were fixed for 1 h at room temperature in 2% glutaraldehyde in 0.1 *M* sodium-cacodylate-buffered seawater, pH 7, containing 0.044 *M* sucrose and 0.01 *M* $CaCl_2$ (modified from Anderson and Personne, 1970). The planulae were rinsed in three changes of this vehicle, for 10 min each, at room temperature. They were then postfixed in 1% osmium tetroxide in 0.1 *M* sodium cacodylate, pH 7, for 1 h and rinsed 30 min in the above buffer. The specimens were dehydrated in an ethanol series, infiltrated with propylene oxide, and embedded in an Epon-Araldite mixture. Specimens so fixed were sectioned with a diamond knife (MJO Company) on a Porter Blum MT2-B ultramicrotome. Sections were placed on copper grids, stained in 3% uranyl acetate (Watson, 1958) followed by 0.2% lead citrate (Veneable and Coggeshall, 1965), and examined with a Zeiss EM9S-2 electron microscope.

Plastic sections 0.18–0.36 μm thick from a region of the block adjacent to that used for thin sections were mounted on glass slides and stained with 0.5% toluidine blue in 1% sodium borate for light microscopy.

Planulae were fixed in 10% formalin in seawater and embedded in Paraplast Plus paraffin. Serial sections 10 μm thick were mounted on glass slides and stained with Azure B for detecting ribonucleic acids (RNA) (Swift, 1955). The cellular compositions of control and treated planulae were determined by counting the number of nuclei in individual cells from serial transverse sections of 82-h planulae. In both control and colchicine-treated planulae, 2000 cells were counted in each of 10 planulae. The number of epitheliomuscle or neurosensory cells (indistinguishable with the method used), mucous cells, interstitial cells, nematoblasts, and ganglionic cells per 20,000 cells was counted for each group. Gastrodermal epithelial cells were not counted.

Results

The 82-h control planula of *Pennaria* displayed an array of highly organized cell types. The ectoderm was composed of epitheliomuscle cells, mucous cells, interstitial cells, nematoblasts, ganglionic cells, and neurosensory cells. The epitheliomuscle cells and mucous cells were abundant by 8 h after fertilization, constituting the majority of the cells in the ectoderm. Interstitial cells and a few forming nematoblasts first appeared in the endoderm between 8–10 h postfertilization. The interstitial cells and the nematoblasts increased in numbers as development proceeded. They eventually migrated to the ectoderm. Ganglionic cells arose at 24 h, whereas neurosensory cells were not present until 48 h after fertilization.

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cells, and form a nerve plexus throughout the length of the planula. N = nucleus of ganglionic cell. Bar = 2 μm.

FIGURE 3. Ciliary rootlet (arrow) of a ganglionic cell. N = nucleus of ganglionic cell. Bar = 1 μm.

TABLE I

Average cell compositions of treated planulae of Pennaria tiarella *(percent of cells) after 72-h recovery. (20,000 cells counted per group.)*

Chemical Treatment	Epitheliomuscle Cells or Neurosensory Cells	Mucous Cells	Interstitial Cells	Nematoblasts	Ganglionic Cells
Control	52	17	10	12	9
Colchicine (0.4%)	80	20	0	0	0

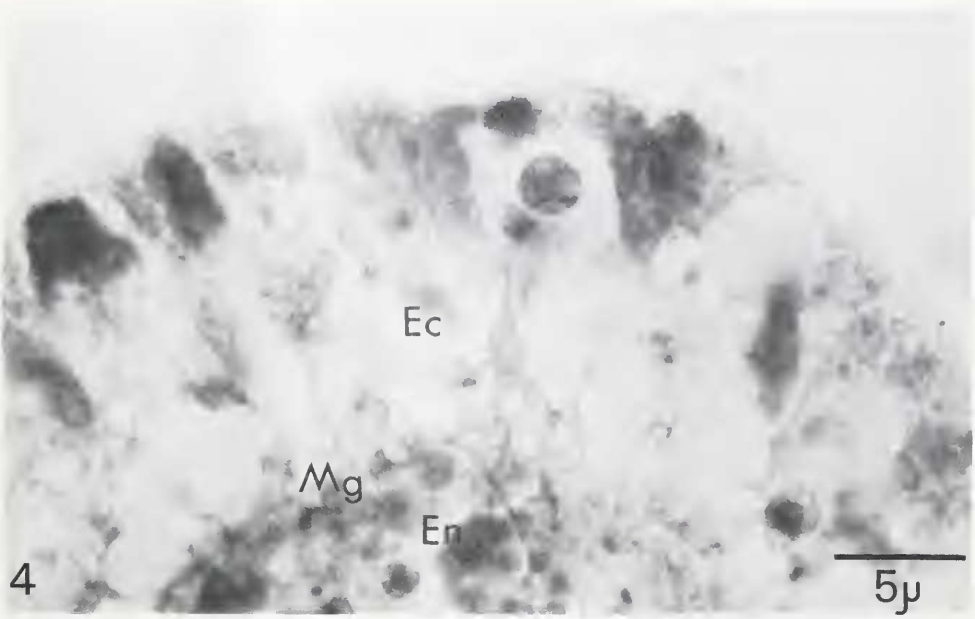
After examining, by light microscopy or TEM, approximately 70 embryos allowed to recover for 12, 24, or 72 h after treatment, we concluded that treatment of 8-h embryos for 2 h with colchicine completely eliminated interstitial cells, nematoblasts, and ganglionic cells (Table I). The elimination of these cells began approximately 22 h after fertilization and was completed by 34 h after fertilization. Table I shows the cellular compositions of control and treated planulae allowed to recover for 72 h (82-h planulae). The numbers are the percentage of each cell type relative to total cells counted. In controls, the interstitial cells, nematoblasts, and ganglionic cells comprised 31% of all cells present. Treatment with colchicine eliminated these cells, and only epithelial cells were found. Ultrastructural examination of these planulae (Fig. 1) repeatedly showed no ganglionic cells. In untreated planulae of comparable age, ganglionic cells and their corresponding neurites were abundant apical to the forming foot processes of the epitheliomuscle cells (Fig. 2). These ganglionic cells possessed a single cilium (Fig. 3) and contained neurosecretory droplets.

Ganglionic cells stain intensely with Azure B (Martin and Thomas, 1980). These Azure B-positive cells were diffusely distributed along the entire length of the larva, and corresponded to the neurite-producing cells seen by transmission electron microscopy. In colchicine-treated planulae the ganglionic cells were absent from transverse serial sections of paraffin-embedded material stained for RNA (Fig. 4). Azure B-positive ganglionic cells were present in corresponding controls (Fig. 5).

We observed neurosensory cells identical to those of control planulae in all the colchicine-treated larvae (Fig. 6). These cells appeared in the ectoderm 48 h after fertilization both in treated planulae devoid of interstitial cells and in control planulae. The cells extended from the free surface of the planula to the mesoglea. They were distinguished from epitheliomuscle cells and mucous cells in that they possessed a single cilium with a complex basal body assembly (Figs. 6 and 7) and numerous microtubules in the surrounding cytoplasm (Fig. 8).

DISCUSSION

Most studies on interstitial cells have been restricted to the freshwater genus *Hydra* (David and Campbell, 1972; David, 1973; Campbell and David, 1974; Marcum and Campbell, 1978). These cells are in the ectoderm of *Hydra* and presumably are the stem cells for all of the animal's nerve elements, nematocytes, and gametes (David and Gierer, 1974). Marcum and Campbell (1978) suggest that since these differentiated and specialized cells do not divide, elimination of the interstitial cells should result in the complete absence of nerve cells and other interstitial cell progeny.



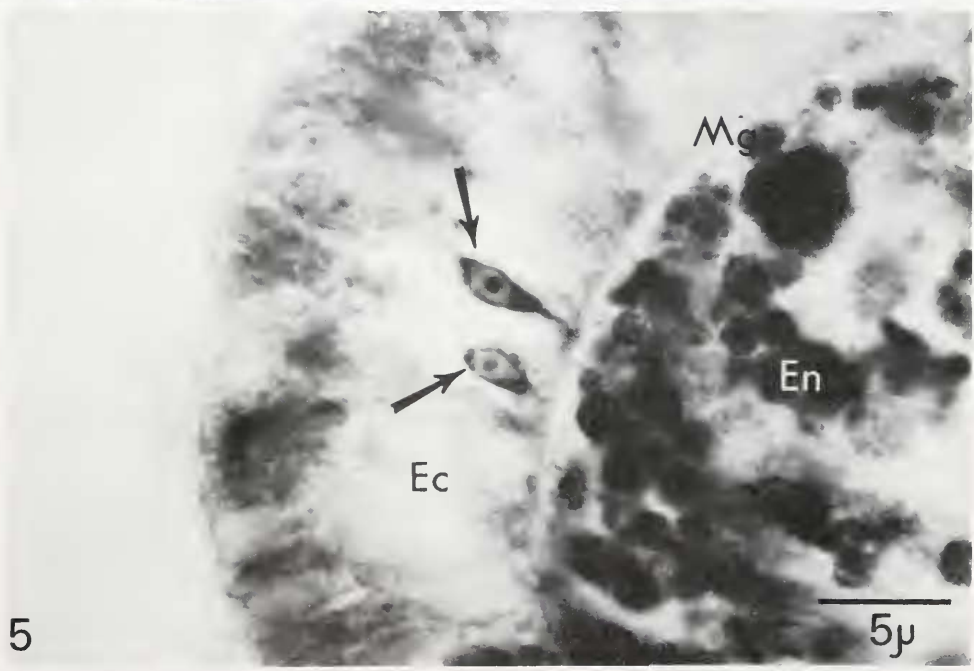


FIGURE 4. Light micrograph of a colchicine-treated planula (stained with Azure B) which has recovered for 72 h. Note the absence of ganglionic cells just apical to the mesoglea. Ec = ectoderm; En = endoderm; Mg = mesoglea. Bar = 5 μm.

FIGURE 5. Light micrograph of a control planula taken from a region comparable to that of Figure 4. Azure B-positive ganglionic cells (arrows) are in an area just above the mesoglea. Note the intense staining of the nucleolus and cytoplasm of these cells. Ec = ectoderm; En = endoderm; Mg = mesoglea. Bar = 5 μm.

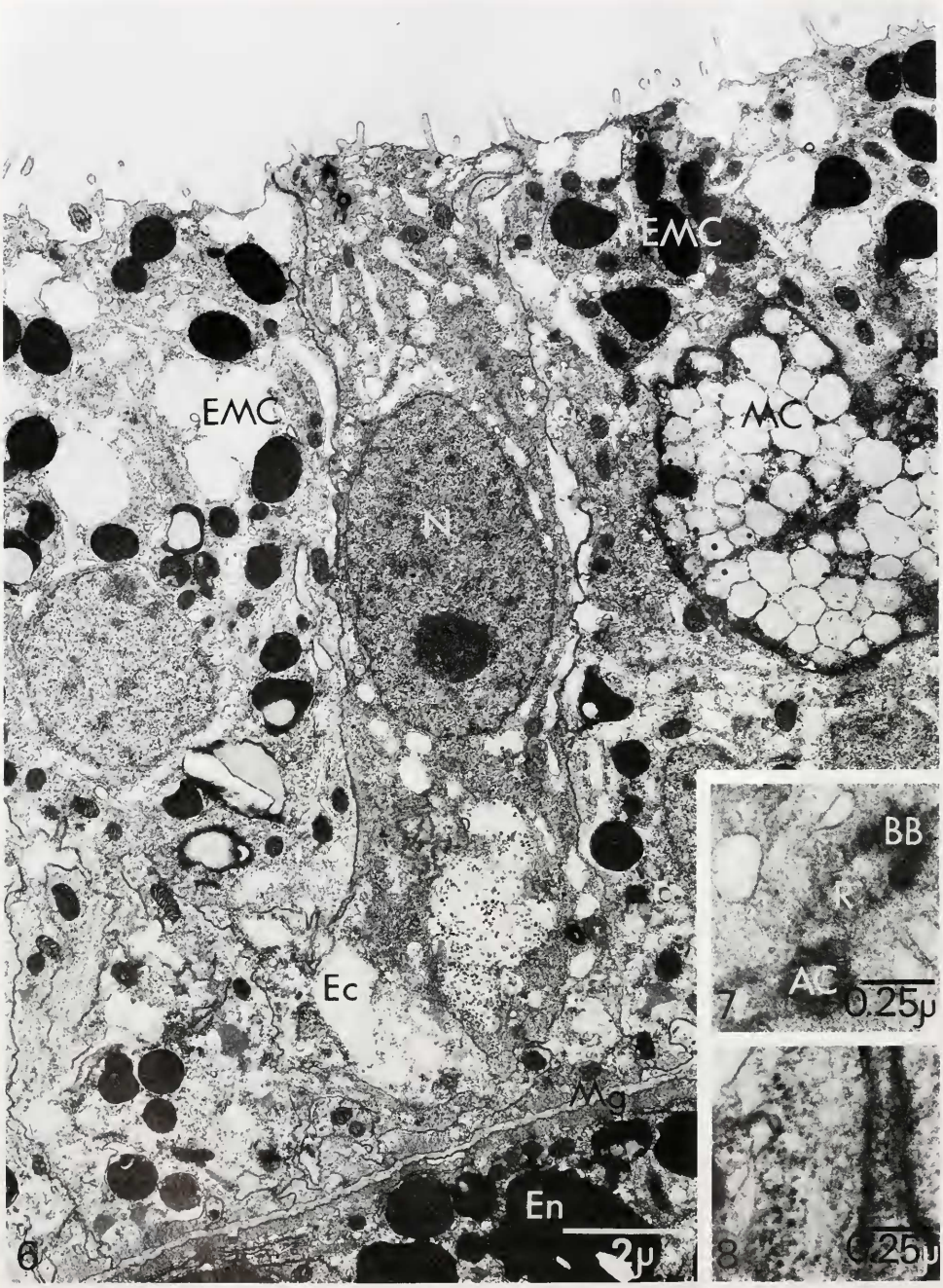


FIGURE 6. Neurosensory cell of a colchicine-treated planula which has recovered for 72 h after treatment. The cell is between two epitheliomuscle cells. Its morphology is identical to that of a neurosensory cell in a control planula. The cell extends from the free surface of the planula to the mesoglea and is characterized by a single cilium with a complex basal body assembly. Ec = ectoderm; EMC = epitheliomuscle cell; En = endoderm; MC = mucous cell; Mg = mesoglea; N = nucleus of neurosensory cell. Bar = 2 μm.

Campbell (1976) and Marcum and Campbell (1978) eliminated interstitial cells from *Hydra* by treating *Hydra* specimens for 8 h in a 0.4% solution of the alkaloid colchicine. The resultant *Hydra* specimens were devoid of interstitial cells, nerve cells, and nematocytes. However, they continued to bud, exhibit tissue renewal patterns, regenerate, and preserve polarity.

Numerous investigators have tried but failed to eliminate interstitial cells from various marine cnidarians (Campbell, personal communication). The elimination of the interstitial cells in 8-h embryos of *Pennaria tiarella* is to our knowledge the first such successful attempt.

In *Pennaria tiarella*, interstitial cells arise 8 h after fertilization from a central core of endodermal cells (Summers and Haynes, 1969; Martin and Thomas, 1977). Summers and Haynes (1969) report that these cells migrate to the ectoderm, where they differentiate into nerves and nematoblasts. Thus they suggest an endodermally-derived nervous system. Two distinct types of nerve cells have been identified with certainty in *Pennaria* (Martin and Thomas, 1980). The present study suggests a dual origin of the nervous system in *Pennaria*. When endodermally-derived interstitial cells were eliminated by colchicine, ganglionic cells were absent, but neurosensory cells were present. Furthermore, these neurosensory cells appeared 48 h postfertilization in planulae that had been rendered free of interstitial cells by 34 h postfertilization. If the interstitial cells were the sole stem cells for the nerve elements, then one would have expected that both ganglionic cells and neurosensory cells would have been eliminated. Thus, the neurosensory cells do not arise from interstitial cells.

We previously reported (Martin and Thomas, 1977) two types of ectodermally-derived cells in *Pennaria*: epitheliomuscle cells and mucous cells. The neurosensory cells must be added to the list. The neurosensory cell arises either from a stem cell of ectodermal origin or from dedifferentiation of epitheliomuscle cells or mucous cells. As we have never seen such a stem cell in the ectoderm, the first possibility seems unlikely. We have, however, observed dividing epitheliomuscle cells that gave rise to mucous cells. If epitheliomuscle cells produce mucous cells, then perhaps they also give rise to the neurosensory cells.

Thus, although ganglionic cells are interstitial cell derivatives, neurosensory cells may arise during larval development in *Pennaria* from some cell altogether different from the interstitial cell. Demonstration of a dual origin of the nervous system, together with the development of a method of eliminating one of the two nerve cell types, opens new avenues for analyzing cell function and intercellular interactions.

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FIGURE 7. Basal body (BB), accessory centriole (AC), and rootlet (R) of a neurosensory cell from a colchicine-treated planula. Bar = 0.25 μm.

FIGURE 8. Microtubules in the apical cytoplasm of a neurosensory cell from a colchicine-treated planula. Bar = 0.25 μm.

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